

WHAT IS CLAIMED:

1. An isolated nucleic acid molecule encoding a mutant phytase,
wherein said mutant phytase either:
 - (i) comprises an amino acid sequence having at least 96 percent
5 sequence identity to SEQ ID NO:2 over a region of at least 100 amino acid residues and
containing at least one substitution of at least one amino acid residue selected from the
group consisting of residue 50, 91, 94, 228, 262, 300, and 301 of SEQ ID NO:2; or
 - (ii) comprises an amino acid sequence having at least 96 percent
sequence identity to SEQ ID NO:4 over a region of at least 100 amino acid residues and
10 containing a substitution of amino acid residue 363 of SEQ ID NO:4.
2. The isolated nucleic acid molecule according to claim 1, wherein
said at least one substitution is of amino acid residue 50 of SEQ ID NO:2 and is selected
from the group consisting of Q50L and Q50P.
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3. The isolated nucleic acid molecule according to claim 1, wherein
said at least one substitution is of amino acid residue 91 of SEQ ID NO:2 and is selected
from the group consisting of K91A and K91E.
- 20 4. The isolated nucleic acid molecule according to claim 1, wherein
said at least one substitution is of amino acid residue 94 of SEQ ID NO:2 and comprises
K94E.
- 25 5. The isolated nucleic acid molecule according to claim 1, wherein
said at least one substitution is of amino acid residue 228 of SEQ ID NO:2 and is selected
from the group consisting of E228Q and E228K.
- 30 6. The isolated nucleic acid molecule according to claim 1, wherein
said at least one substitution is of amino acid residue 262 of SEQ ID NO:2 and comprises
D262H.

7. The isolated nucleic acid molecule according to claim 1, wherein said at least one substitution is of amino acid residue 300 of SEQ ID NO:2 and is selected from the group consisting of K300R, K300T, K300D, and K300E.

5 8. The isolated nucleic acid molecule according to claim 1, wherein said at least one substitution is of amino acid residue 301 of SEQ ID NO:2 and comprises K301E.

9. The isolated nucleic acid molecule according to claim 1, wherein
10 said substitution of amino acid residue 363 of SEQ ID NO:4 is M363L.

10. The isolated nucleic acid molecule according to claim 1, wherein said at least one substitution comprises a double-substitution.

15 11. The isolated nucleic acid molecule according to claim 10, wherein said double-substitution is selected from the group consisting of K300E/K301E, K300D/E228K, K300T/E228K, K300R/E228K, and E228K/K94E.

12. The isolated nucleic acid molecule according to claim 1, wherein
20 said at least one substitution comprises a triple-substitution.

13. The isolated nucleic acid molecule according to claim 12, wherein said triple-substitution is selected from the group consisting of K300R/K301E/E228K, K300T/K301E/E228K, K300D/K301E/E228K, K300E/K301E/K94E,
25 K301E/E228K/K94E, and K300E/K91A/E228Q.

14. The isolated nucleic acid molecule according to claim 1, wherein said at least one substitution comprises a quadruple substitution.

30 15. The isolated nucleic acid molecule according to claim 14, wherein said quadruple-substitution comprises K300D/K94A/E228A/D262A.

16. A recombinant DNA expression system comprising a nucleic acid molecule according to claim 1.

17. The expression system according to claim 16, wherein the nucleic acid molecule is in a heterologous expression vector.

18. A host cell comprising a heterologous nucleic acid molecule according to claim 1.

19. The host cell according to claim 18, wherein said host cell is a yeast cell.

20. The host cell according to claim 19, wherein the yeast cell is of a strain selected from the group consisting of *Saccharomyces*, *Kluyveromyces*, *Torulaspora*, *Schizosaccharomyces*, *Pichia*, *Hansenula*, *Torulopsis*, *Candida*, and *Karwinskia*.

21. The host cell according to claim 19, wherein the yeast cell is a methylotrophic yeast strain.

22. The host cell according to claim 21, wherein the methylotrophic yeast strain is selected from the group consisting of *Pichia*, *Hansenula*, *Torulopsis*, *Candida*, and *Karwinskia*.

23. The host cell according to claim 18, wherein said host cell is a non-yeast cell.

24. The host cell according to claim 23, wherein said non-yeast cell is selected from the group consisting of *Aspergillus* species, *Trichoderma* species, and *Neurospora* species.

25. A method of recombinantly producing a mutant phytase
comprising:

transforming a host cell with at least one heterologous nucleic acid
molecule according to claim 1 under conditions suitable for expression of the mutant
5 phytase; and
isolating the mutant phytase.

26. The method according to claim 25, wherein the host cell is a yeast
cell.

27. The method according to claim 26, wherein the yeast cell is of a
strain selected from the group consisting of *Saccharomyces*, *Kluyveromyces*,
Torulaspora, *Schizosaccharomyces*, *Pichia*, *Hansenula*, *Torulopsis*, *Candida*, and
Karwinskia.

28. The method according to claim 26, wherein the yeast cell is a
methylophilic yeast strain.

29. The method according to claim 28, wherein the methylophilic
20 yeast strain is selected from the group consisting of *Pichia*, *Hansenula*, *Torulopsis*,
Candida, and *Karwinskia*.

30. The method according to claim 25, wherein said host cell is a non-
yeast cell.

31. The method according to claim 30, wherein said non-yeast cell is
selected from the group consisting of *Aspergillus* species, *Trichoderma* species, and
Neurospora species.

32. An isolated mutant phytase comprising either:
- (i) an amino acid sequence having at least 96 percent sequence identity to SEQ ID NO:2 over a region of at least 100 amino acid residues and containing at least one substitution of at least one amino acid residue selected from the group consisting of residue 50, 91, 94, 228, 262, 300, and 301 of SEQ ID NO:2; or
 - (ii) an amino acid sequence having at least 96 percent sequence identity to SEQ ID NO:4 over a region of at least 100 amino acid residues and containing a substitution of amino acid residue 363 of SEQ ID NO:4.
33. The isolated mutant phytase according to claim 32, wherein said isolated mutant phytase is in pure or non-pure form.
34. The isolated mutant phytase according to claim 32, wherein said isolated mutant phytase is recombinant.
35. The isolated mutant phytase according to claim 32, wherein said at least one substitution is of amino acid residue 50 of SEQ ID NO:2 and is selected from the group consisting of Q50L and Q50P.
36. The isolated mutant phytase according to claim 32, wherein said at least one substitution is of amino acid residue 91 of SEQ ID NO:2 and is selected from the group consisting of K91A and K91E.
37. The isolated mutant phytase according to claim 32, wherein said at least one substitution is of amino acid residue 94 of SEQ ID NO:2 and comprises K94E.
38. The isolated mutant phytase according to claim 32, wherein said at least one substitution is of amino acid residue 228 of SEQ ID NO:2 and is selected from the group consisting of E228Q and E228K.
39. The isolated mutant phytase according to claim 32, wherein said at least one substitution is of amino acid residue 262 of SEQ ID NO:2 and comprises D262H.

40. The isolated mutant phytase according to claim 32, wherein said at least one substitution is of amino acid residue 300 of SEQ ID NO:2 and is selected from the group consisting of K300R, K300T, K300D, and K300E.

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41. The isolated mutant phytase according to claim 32, wherein said at least one substitution is of amino acid residue 301 of SEQ ID NO:2 and comprises K301E.

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42. The isolated mutant phytase according to claim 32, wherein said substitution of amino acid residue 363 of SEQ ID NO:4 is M363L.

43. The isolated mutant phytase according to claim 32, wherein said at least one substitution comprises a double-substitution.

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44. The isolated mutant phytase according to claim 43, wherein said double-substitution is selected from the group consisting of K300E/K301E, K300D/E228K, K300T/E228K, K300R/E228K, and E228K/K94E.

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45. The isolated mutant phytase according to claim 32, wherein said at least one substitution comprises a triple-substitution.

46. The isolated mutant phytase according to claim 45, wherein said triple-substitution is selected from the group consisting of K300R/K301E/E228K, K300T/K301E/E228K, K300D/K301E/E228K, K300E/K301E/K94E, K301E/E228K/K94E, and K300E/K91A/E228Q.

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47. The isolated mutant phytase according to claim 32, wherein said at least one substitution comprises a quadruple-substitution.

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48. The isolated mutant phytase according to claim 47, wherein said quadruple-substitution comprises K300D/K94A/E228A/D262A.

49. An animal feed composition comprising the isolated mutant phytase according to claim 32.

5 50. A foodstuff comprising an animal feed composition according to claim 49.

51. The foodstuff according to claim 50, wherein the foodstuff further comprises greater than 1.0% by weight vitamin and mineral mix.

10 52. The foodstuff according to claim 50, wherein the foodstuff further comprises soybean meal.

15 53. The foodstuff according to claim 50, wherein the foodstuff further comprises antibiotics.

54. A method of feeding a monogastric animal comprising:
feeding to the animal a foodstuff in combination with the isolated mutant phytase according to claim 32.

20 55. The method according to claim 54, wherein the animal is a fowl species.

25 56. The method according to claim 54, wherein the animal is a porcine species.

57. The method according to claim 54, wherein the animal is an aquatic species.

30 58. The method according to claim 54, wherein the animal is a domestic animal selected from the group consisting of a canine species and a feline species.

59. The method according to claim 54, wherein the animal is a mammalian species selected from the group consisting of an *Oryctolagus* species, a *Capra* species, a *Bos* species, an *Equus* species, and an *Ovis* species.

5 60. The method according to claim 54, wherein there are about 100-2,000 units of the mutant phytase per kilogram of the foodstuff.

61. The method according to claim 54, wherein the mutant phytase has an altered pH profile and an altered pH optima compared to a corresponding non-
10 mutant phytase.

62. A method of improving the nutritional value of a foodstuff consumed by an animal, said method comprising:
providing a foodstuff comprising *myo*-inositol hexakisphosphate;
15 providing a mutant phytase according to claim 32; and
feeding to the animal the foodstuff in combination with the mutant phytase under conditions effective to increase the bioavailability of phosphate from phytate.

63. The method according to claim 62, wherein the animal is poultry.
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64. The method according to claim 62, wherein the animal is a porcine species.

65. The method according to claim 62, wherein the animal is an
25 aquatic species.

66. The method according to claim 62, wherein the animal is a domestic animal selected from the group consisting of a canine species and a feline species.
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67. The method according to claim 62, wherein the animal is a mammalian species selected from the group consisting of an *Oryctolagus* species, a *Capra* species, a *Bos* species, an *Equus* species, and an *Ovis* species.

68. The method according to claim 62, wherein the animal is a human.

69. The method according to claim 62, wherein the foodstuff is pig
5 feed.

70. The method according to claim 62, wherein the foodstuff is poultry
feed.

10 71. The method according to claim 62, wherein the animal is fed the
foodstuff in combination with about 100-2,000 units of the mutant phytase per kilogram
of the foodstuff.

72. A method for altering the enzymatic properties of a wild-type
15 phytase of an *Aspergillus* species, said method comprising:
providing a wild-type phytase of an *Aspergillus* species selected from the
group consisting of *Aspergillus niger* and *Aspergillus fumigatus*, wherein said *Aspergillus*
niger wild-type phytase comprises an amino acid sequence having at least 96 percent
sequence identity to SEQ ID NO:2 over a region of at least 100 amino acid residues, and
20 wherein said *Aspergillus fumigatus* wild-type phytase comprises an amino acid sequence
having at least 96 percent sequence identity to SEQ ID NO:4 over a region of at least 100
amino acid residues; and

altering the amino acid sequence of said wild-type phytase under
conditions effective to yield a mutant phytase having a modified substrate binding region
25 and/or improved catalytic efficiency compared to the amino acid sequence of said wild-
type phytase, wherein said altering comprises either:

- (i) introducing into the amino acid sequence of said *Aspergillus niger*
wild-type phytase at least one substitution of at least one amino acid residue selected from
the group consisting of residue 50, 91, 94, 228, 262, 300, and 301 of SEQ ID NO:2; or
- 30 (ii) introducing into the amino acid sequence of said *Aspergillus*
fumigatus wild-type phytase a substitution at amino acid residue 363 of SEQ ID NO:4.

73. The method according to claim 72, wherein said at least one substitution is of amino acid residue 50 of SEQ ID NO:2 and is selected from the group consisting of Q50L and Q50P.

5 74. The method according to claim 72, wherein said at least one substitution is of amino acid residue 91 of SEQ ID NO:2 and is selected from the group consisting of K91A and K91E.

75. The method according to claim 72, wherein said at least one
10 substitution is of amino acid residue 94 of SEQ ID NO:2 and comprises K94E.

76. The method according to claim 72, wherein said at least one substitution is of amino acid residue 228 of SEQ ID NO:2 and is selected from the group consisting of E228Q and E228K.

15 77. The method according to claim 72, wherein said at least one substitution is of amino acid residue 262 of SEQ ID NO:2 and comprises D262H.

78. The method according to claim 72, wherein said at least one
20 substitution is of amino acid residue 300 of SEQ ID NO:2 and is selected from the group consisting of K300R, K300T, K300D, and K300E.

79. The method according to claim 72, wherein said at least one substitution is of amino acid residue 301 of SEQ ID NO:2 and comprises K301E.
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80. The method according to claim 72, wherein said substitution of amino acid residue 363 of SEQ ID NO:4 is M363L.

81. The method according to claim 72, wherein said at least one
30 substitution comprises a double-substitution.

82. The method according to claim 81, wherein said double-substitution is selected from the group consisting of K300E/K301E, K300D/E228K, K300T/E228K, K300R/E228K, and E228K/K94E.

5 83. The method according to claim 72, wherein said at least one substitution comprises a triple-substitution.

84. The method according to claim 83, wherein said triple-substitution is selected from the group consisting K300R/K301E/E228K, K300T/K301E/E228K,
10 K300D/K301E/E228K, K300E/K301E/K94E, K301E/E228K/K94E, and K300E/K91A/E228Q.

85. The method according to claim 72, wherein said at least one substitution comprises a quadruple-substitution.

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86. The method according to claim 85, wherein said quadruple-substitution comprises K300D/K94A/E228A/D262A.

87. A method of *in vitro* hydrolysis of phytate, said method
20 comprising:
providing a mutant phytase according to claim 32 and
combining said mutant phytase with a phytate source under conditions effective to increase the bioavailability of phosphate from said phytate source.

25 88. The method according to claim 87, wherein said phytate source is an animal feed.

89. The method according to claim 87, wherein said phytate source is a foodstuff.

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90. The method according to claim 87 further comprising combining said mutant phytase with a phytate source under conditions effective to increase the bioavailability from said phytate source of minerals selected from the group consisting of calcium, zinc, and iron.

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91. A method of improving the nutritional value of a foodstuff consumed by humans, said method comprising:

providing a mutant phytase according to claim 32 and

combining said mutant phytase with a foodstuff consumed by humans

10 under conditions effective to increase the bioavailability of minerals from said foodstuff, wherein said minerals are selected from the group consisting of iron, zinc, phosphorus, and calcium.

92. A method of imparting improved mineral nutritional value to a
15 plant that is edible for consumption by animals, said method comprising:

providing a transgene comprising an isolated nucleic acid molecule according to claim 1 operatively associated with a regulatory sequence containing transcriptional and translational regulatory elements that control expression of the isolated nucleic acid molecule in a transgenic plant cell;

20 providing a non-transformed plant that is edible for consumption by animals; and

inserting the transgene into the genome of the non-transformed plant under conditions effective to yield a transformed plant that transgenically expresses a mutant phytase encoded by the isolated nucleic acid molecule, wherein said transformed plant
25 has improved mineral nutritional value compared to that of said non-transformed plant.